EVALUATION OF PULSED-FIELD GEL ELECTROPHORESIS' ABILITY TO CLUSTER CAMPYLOBACTER JEJUNI BY ANIMAL EXPOSURE OR LOCATION

by

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ABSTRACT

Campylobacter has emerged in the last 30 years as the most common source of gastroenteritis worldwide. Considering *Campylobacter's* vast ubiquity, surprisingly little is known regarding its source. Most cases are deemed sporadic and few outbreaks are recognized. Pulsed-field gel electrophoresis (PFGE) data collected at the Unified State Laboratories: Public Health since 2002 was combined with epidemiological data containing patient county of residence and reported animal exposure. In total, 1,728 PFGE patterns were analyzed, 1,538 of these contained county of residence data and 718 included animal exposure data. This information was analyzed together and trends examined. Counties with a higher population and larger animal groups exhibited a lower PFGE pattern variation. Certain common PFGE patterns were also found to be more prevalent among different counties and animal groups. Continued analysis is needed to better understand *Campylobacter* infection and prevent additional cases.

This thesis is dedicated to the best parents on Earth, Sue and Charlie Wagner,

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CHAPTER I

INTRODUCTION

In 1886, Theodor Escherich discovered what is now thought to have been *Campylobacter* species in stool samples taken from infants with diarrhea who had died. He referred to those offending organisms as ‘cholera infantum’ (1, 14, 45). In 1906, veterinarians McFadyean and Stockman experienced a spike in abortion rates among ewes and first isolated what we now know to be *Campylobacter* from uterine mucus samples. Years later in 1919, Americans Theobald Smith and Marian Taylor isolated an identical organism from aborted calves. After familiarizing themselves with the work done by McFadyean and Stockman, they confirmed these organisms to be identical and named the organism ‘*Vibrio fetus*’ (9, 20, 30, 44).

Thus far *Campylobacter* organisms had been isolated only from animals. The first human association occurred in 1947 when three pregnant women presented with *Vibrio fetus* in their blood, causing two to abort (9, 20, 30). Years later the first human *Campylobacter* was cultured from blood involving an outbreak of diarrhea in children. In 1957, Elizabeth King distinguished two groups of organisms in this outbreak and referred to them as ‘related *Vibrios*’ and

believed the others to be similar to *Vibrio fetus*. She was the first to bring attention to its effect on humans in the late 1950s (1, 9, 20, 30, 42).

Finally in 1963, the genus *Campylobacter* was proposed after scientists realized the large genetic and metabolic differences to that of *Vibrios* (20, 30). Following the reclassification, King's research along with Dekeyser and Butzler's laboratory methods allowed *Campylobacter* to be isolated from a young woman's stool in 1972 (1, 9, 30, 42). This laboratory filtration method enabled the source of *Campylobacter* infection to be determined and therefore better understood. Since then, selective medias have been developed, enabling direct culture from stool, bringing *Campylobacter* to the forefront of enteric disease since the 1980s (1, 9, 20).

Bacteriology

In 1963, scientists Sebald and Véron proposed the term *Campylobacter*, meaning a curved rod, to rightfully distinguish these from *Vibrios* (20, 42). *Campylobacter jejuni* is only one of the species recognized in the family Campylobacteraceae. In total, the genus Campylobacter includes 17 species, six subspecies, and two biovars (22, 23, 34). Species *Campylobacter jejuni* and *Campylobacter coli* represent 80 to 85% and 10 to 15% respectively of all human *Campylobacter* infections (30).

Viewed under the microscope, *Campylobacter* have a curved or spiral-shaped rod appearance and are likened to gulls-wings. *Campylobacter* are comparatively small, ranging in size from 0.2 to 0.5 µm wide by 0.5 to 8 µm long

and have polar unsheathed flagellae at either one or both ends. These flagella enable the organism to be highly motile with characteristic rapid, darting, corkscrew-like motility. This motility has proven to be one of *Campylobacter's* most important virulence factors. *Campylobacter* are gram-negative, non-spore-forming, microaerophilic bacteria that grow best in atmospheres containing 5% oxygen, 10% carbon dioxide, and 85% nitrogen (14, 20, 45). *Campylobacter jejuni* grows best at 42ºC while all *Campylobacter* species will grow at 32ºC (20). Biochemical activity is lacking in *Campylobacter* as they do not ferment or oxidize carbohydrates causing laboratory speciation methods to be cumbersome and therefore rarely utilized (14, 45).

Pathogenesis

Campylobacter has emerged in the last 30 years to become the leading cause of gastroenteritis worldwide. Yearly infections range from 2 to 3 million cases of *Campylobacter* in the United States alone (1, 23, 34, 45). These numbers represent only confirmed cases, which are thought to be 8 to 30 times less than the true population incidence (24). As *Campylobacter* is not a reportable disease in many states, the true infectivity is largely unknown. Overall, the incidence has increased so much that in developed countries, *Campylobacter* infection accounts for more illness than *Shigalla* and *Salmonella* species combined (28). Because *Campylobacter* infections are usually self-limiting, few people actually visit their doctor and submit a stool sample that is required to confirm a *Campylobacter* infection.

Humans are infected with *Campylobacter* orally with an infectious dose of at least 500 organisms (14, 24). Once exposed, the incubation period usually lasts 2-3 days. Symptoms of diarrhea, fever, abdominal cramps, and nausea can occur and last up to 7 days (30, 45). In some more severe cases and more commonly seen in children, diarrhea can be bloody (23). It is not uncommon for *Campylobacter* infection to begin with a prodrome of influenza-like illness excluding diarrhea, typically indicative of a more severe disease. Following resolution of symptoms, humans can shed *Campylobacter* in their stool for several weeks (24).

The fastidious nature of these bacteria makes it difficult to understand their prevalence. Surprisingly, *Campylobacter* is very sensitive to environmental stresses such as dryness, heat, and overexposure to oxygen (35). Several virulence factors have been identified attributing to *Campylobacter's* disease burden. Motility, chemotaxis, adherence mechanisms, and the ability to produce toxins are thought to be the main methods by which *Campylobacter* induces disease (4, 14, 24). After ingestion, a large percentage of organisms are killed in the acidic environment of the stomach (24). Those that survive move onto the ileum and jejunum to colonize, and then move to target the colon (34, 42). An inflammation response in the lower bowel causes many patients to have blood, pus, or mucus in their stool (9, 47).

Although *Campylobacter* infection is usually self-limiting, more severe cases or immunocompromised patients may benefit from antimicrobial therapy early in the course of disease (9). Most cases require only supportive care, such

as fluid and electrolyte replacement (1, 9, 30). Research has shown that the severity of disease is strain specific as well as dose dependent (1, 24, 47). Several serious sequelae have been attributed to *Campylobacter* including Guillain-Barré syndrome (GBS), Miller Fisher syndrome, Rieter syndrome, reactive arthritis, irritable bowel syndrome, urticaria, and erythema nodosum (4, 23, 30). The most serious of these has been determined to be GBS with an estimated occurrence of 1:1000 following *Campylobacter* infection (9, 23). *Campylobacter,* Penner type HS:19, is more likely to cause GBS with an elevated risk of 1:200 infections (23). Among those diagnosed with GBS, 30 to 50% have had a fairly recent history of *Campylobacter* infection (30). It is thought that the molecular structure of *Campylobacter* mimics that of human nervous tissue leading antibodies to cross-react and cause GBS (23). These severe consequences strengthen the need for a better understanding of transmission and therefore prevention.

Epidemiology

Although *Campylobacter* is mainly thought of as a foodborne disease, other risk factors include drinking raw milk or contaminated water, foreign travel, and contact with pets and other animals. *Campylobacter* is a zoonotic pathogen and has been isolated from most animals, both wild and domestic, that are mainly asymptomatic carriers (9, 24, 28, 46). Animals constitute *Campylobacter's* main reservoir and transmit either directly or indirectly to humans where the

transmission typically ends after approximately one week of illness. Person-to-person transmission is not common and usually involves young children (43).

Much concern has been placed on foods of animal origin as a major cause of human *Campylobacter* infections. Poultry is accepted as the most common source of infection worldwide. This role was documented in 1999 when Belgium withdrew poultry and eggs from store shelves because of dioxin poisoning. In the subsequent months, *Campylobacter* infections dropped 40% and returned to normal levels once the ban was lifted (23, 30). Research shows that up to 60% of raw chickens entering households are infected with *Campylobacter,* and that the organism load could deliver several infectious doses (23). This statistic is not altogether surprising as birds provide the perfect atmosphere for thermophilic *Campylobacter* with their metabolic temperature equaling that of *Campylobacter's* optimal growth temperature of 42ºC (1, 22). It is commonly thought that wild birds are the main natural reservoir from which most all other animals obtain *Campylobacter*, ultimately leading to human infections (1, 22, 23, 43).

Campylobacter's ubiquity is proven by the huge range of species it is able to infect and oftentimes carry asymptomatically (20, 21). In industrialized countries today, more people commonly associate with household pets rather than farm animals. In these circumstances, *Campylobacter* infection can be largely attributed to dogs and cats, especially the young and those suffering from diarrhea (21, 43). As domestic animals have become "family members" this increased proximity to humans provides more opportunities for infection.

Veterinarians, farmers, and those associated with meat processing also have a higher risk of contracting *Campylobacter* through occupational direct contact (43). Although *Campylobacter* is a commensal organism of livestock, cattle and sheep have been shown to intermittently shed *Campylobacter*. Colonization is more common in younger animals, those contained in feedlots, and in those undergoing stressful situations such as transport, birthing, and slaughter (1, 20, 23, 28, 30).

Several studies have shown that the environment plays a critical role in *Campylobacter* infection of animals (23). Wild birds contaminate natural waters with fecal material, and *Campylobacter* is able to survive very cold temperatures in water for several weeks. These factors increase the risk of animals being exposed to *Campylobacter* (43). *Campylobacter* species are unique in their ability to enter into a dormant form when confronted with stresses in their environment. This survival mode, referred to as “viable, but nonculturable” phase, allows the organism to retain their viability until exposed to more favorable conditions. This ability contributes greatly to *Campylobacter’s* prevalence in the environment. Unfortunately, while *Campylobacter* resides in its dormant form and is nonculturable, laboratory methods of detection are futile. When dormant, *Campylobacter* changes from a spiral shape to a coccoid form. This form allows the organism to basically hibernate in the environment until conditions change and become more suitable for growth and transmission (23, 38).

Favorable growth conditions for *Campylobacter* in temperate climates translate into almost twice the number of infections in the summer opposed to wintertime (9). During winter, the coccoid forms of *Campylobacter* linger in streams and slow moving, low oxygenated aquifers until spring arrives, bringing warmer temperatures and inducing metabolic activity. *Campylobacter* incidence peaks in late spring/early summer and again in autumn leading researchers to create correlations between ambient temperature and *Campylobacter* cases (1, 23, 26, 28, 38). *Campylobacter's* growth and survival mechanisms may not be all to blame for the seasonality of cases. Increased ambient temperature can influence behaviors that lead to *Campylobacter* infections such as elevated consumption of raw and undercooked foods and increased exposure to contaminated water while enjoying the outdoors (26).

In developing countries, much of life occurs outdoors alongside domesticated animals and untreated water. Good hygiene and sanitation practices can also be lacking in rural settings, resulting in higher exposure rates (11, 24). Occupations such as dairy farmers, poultry abattoirs, and veterinarians have increased rates of *Campylobacteriosis* due to their proximity to the animal reservoirs (30). In these types of agricultural settings, the prevalence of *Campylobacteriosis* multiplies as do the chances of repeat infections. Rural communities with several risk factors have a different epidemiology than urban settings. This difference is noted by the sharp decline in *Campylobacter* enteritis after childhood in developing countries, opposed to a more gradual decrease followed by another peak at adolescence in industrialized countries (24).

Although infection is highest in young children around the world, developing countries have a much higher prevalence of *Campylobacter*-specific serum antibodies in children than in the United States (2, 6, 24). These antibodies gained following multiple *Campylobacter* infections help children to remain asymptomatic, protecting against disease but not colonization (6, 9, 10, 24). Research done in the Netherlands has shown multiple *Campylobacter* infections during childhood promote immune response-eliciting events that largely decrease the severity of symptoms when exposed to *Campylobacter* again later in life (2, 10). Other research has detected serum and salivary IgG antibodies up to one year following infection and even witnessed an increase in antibodies in some patients over time (10). Conversely, an obvious lack of immunity among the elderly and those with impaired immune systems increases the risk of developing a severe infection (30).

Several studies have been performed to better understand the role immunity, dose-response, and gender plays in *Campylobacteriosis*. Passive immunity has been demonstrated among infants whose mothers secreted *Campylobacter* –specific IgA antibodies in their breast milk and colostrum, resulting in a lower incidence of *Campylobacter* –associated diarrhea (52). Acquired immunity has been demonstrated among chronic raw milk consumers who have increased levels of antibodies compared to those who are naïve to raw milk. Several studies suggest long-lasting immunity following various infection scenarios (6, 10, 30, 52). Another study from the Netherlands cautions against assuming a low risk associated with a low dose of *Campylobacter*. The study

also promoted caution when reviewing volunteer studies suggesting that illness risk is lowest at high doses because the studies lacked information on patients' histories with *Campylobacter* infection (51). An increased incidence of *Campylobacter* in males is not understood. Explanations ranging from poor kitchen techniques to a higher susceptibility have been suggested to account for this increased risk (28, 48).

Answers to the many questions still surrounding *Campylobacter* have been sought through genetics. In 2000, the genome sequence of *C. jejuni* NCTC 11168 was deciphered (19). This information has been imperative to understanding *Campylobacter's* emergence as the most common cause of gastroenteritis worldwide. Unique features including a relatively small genome size (~1,650,000 base pairs), large proportion of A+T base pairs (~70%), very few repeat sequences, and a complete lack of insertion sequence elements, transposons, retron elements, prophages, and plasmids cause *Campylobacter* to distinguish itself from other enteric diseases (13, 19, 32). Although *Campylobacter* does not possess many of the virulence factors of other pathogens like *Salmonella* and toxin-producing *Escherichia coli*, it remains an unusually diverse organism (19, 23). Many studies have shown *Campylobacter's* genome to be highly diverse with about 22 variable regions (34). This heterogeneity has been attributed to genetic material exchange *in vivo* and to widespread genetic chromosomal rearrangements (4). Other methods by which *Campylobacter* has become so diverse are through DNA inversion, horizontal gene transfer, natural transformation, and gene deletions and insertions (4, 46).

This hypervariability seems to ignite with changes in temperature between 37 to 42ºC and during transmission between different animal sources (4).

Unfortunately, a nonprimate animal model that mimics human infection does not exist. This complicates the study of *Campylobacter* as researchers are limited to in vitro cell culture methods and experimental human testing (12, 24, 34, 47). Promising progress has been made on *Campylobacter* vaccines for animals but none has yet to come on the market (9). The unusual genomic variability of *Campylobacter* may hinder the progress of vaccination (24). Methods for better detection, isolation, and prevention are imperative in reversing the emergence of *Campylobacter* seen in the last 30 years.

Laboratory Testing Methods

With *Campylobacter's* emergence in the late 1970s and 1980s, serotyping schemes were necessary to characterize isolates. Penner and Lior serotyping was established in 1980 and 1982, respectively, using heat-stable (HS) and heat-labile (HL) serotyping schemes developed by each. The Penner HS antigen typing scheme detects more than 60 serotypes while the Lior HL antigen typing scheme recognizes more than 100 serotypes (13). Unfortunately, this method, along with biotyping and phage typing requires many reagents, is expensive and has a large proportion of untypeable strains. Although useful, these methods are very labor intensive and rarely utilized (1, 13, 14, 15, 17). Reference laboratories typically identify *Campylobacter* spp. by examining colony characteristics, microaerobic growth at 25ºC, 37ºC, and 42ºC, Gram staining, oxidase tests,

catalase tests, hippurate hydrolysis, nitrate reduction, and oxygen tolerance (9, 14, 41). Commercially available diagnostic tests for *Campylobacter* spp. are also commonly utilized. These tests fall into two categories: antigen and DNA detection systems (14, 30). These diagnostic tests are able to identify organisms directly from clinical specimens or from already isolated cultures. Results are useful for preliminary screening of specimens but are less sensitive and specific compared to culture (14).

Many DNA-based typing methods have been developed because of the issues involved in serotyping *Campylobacter*. For example, genotyping methods such as pulsed-field gel electrophoresis (PFGE), ribotyping, PCR-based methods, and DNA sequencing-based typing have emerged. These methods have been useful in the application of microbial source tracking, where the origin of bacteria is determined by comparing isolates from patients to those of the exposure (29). Although no "gold standard" for *Campylobacter* typing has been identified, PFGE has proven to be a highly discriminatory and useful epidemiological tool (3, 14, 16, 17, 31).

During outbreak investigations, PFGE is considered the most useful and discriminatory method for determining an outbreak's source (3, 25, 27, 36, 40, 41, 50). Because *Campylobacter* is so genetically diverse, restriction fragment length polymorphism assays like PFGE are able to distinguish between epidemiologically-related and nonrelated sources. This degree of genetic diversity has been scrutinized as possibly too diverse, but many studies have found PFGE patterns that have remained stable throughout passage from one

host to another making it very useful in outbreak situations (27, 39). It is not uncommon to have several different strains involved in large environmental *Campylobacter* outbreaks, which is understandable considering the sources (7, 46). Random genetic defects including point mutations, insertions, and deletions of DNA may also alter patterns over time during an outbreak (3, 50).

PFGE is extremely useful as it is able to digest genomic-sized DNA with rare-cutting enzymes and generate a pattern that can be analyzed following electrophoresis. First, an isolated strain of *Campylobacter* is immobilized in agarose forming what is called a plug. Next, this plug is immersed in lysis buffer to force open the cell wall and expose the DNA, and washed to remove all other cellular debris. The plug is restricted using a rare restriction enzyme, typically SmaI, and added to an agarose gel that is subjected to electrophoresis. Because the DNA fragments are so large, traditional electrophoresis is inadequate. PFGE is required to pull the large fragments through the agarose by switching the current's polarity and run time. Once electrophoresis is finished, the gel is stained with a nucleic acid gel stain and visualized using ultraviolet light (18). Differences in these patterns represent changes in the genetic makeup of an organism and can signify relatedness among strains or species.

Foodborne disease surveillance was revolutionized in 1996 with the development of the national molecular subtyping network, PulseNet. The National Food Safety Initiative was developed following President Clinton's announcement in 1997, initiating a nationwide early warning system for foodborne diseases. PulseNet responded to this initiative and set up an

electronic network linking public health laboratories and the Centers for Disease Control and Prevention (CDC) centralized in Atlanta (5). PulseNet combines forces with public health laboratories from all 50 states, the laboratories of the United States Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS), the United States Food and Drug Administration laboratories in the Center for Food Safety and Applied Nutrition (FDA-CFSAN), and Center for Veterinary Medicine (17, 49). In 2001, a rapid and standardized protocol for *Campylobacter* was developed, and a national database soon followed (15). This database allows interlaboratory comparisons to be made, and therefore, multi-state outbreaks to be more easily recognized that may have otherwise gone undetected (40, 49). PulseNet has encouraged and developed close collaboration between microbiologists and epidemiologists that had been lacking before its implementation (49).

Each PulseNet participating laboratory must be equipped with the same mechanics and software. PulseNet's data are extremely reliable because of the strict adherence to standardized protocols, high reproducibility of DNA patterns, and robustness of the analytical software BioNumerics. A CHEF Mapper system is used to perform PFGE because it has flexible electrophoretic conditions and nonlinear ramping that produce better band separation. Once a tagged image file format (TIFF) image has been produced of a gel, it is then imported into BioNumerics. Each gel is then normalized allowing the patterns to be compared to any others, even those generated at different laboratories. Once a laboratory is certified in PFGE, patterns generated at that laboratory can be directly

uploaded from BioNumerics to the national PFGE database located on a PulseNet server at CDC. Here, all uploaded PFGE patterns can be analyzed using BioNumerics by a member of CDC's PulseNet staff. Combining PulseNet data together with traditional epidemiologic methods has allowed assessment of more outbreaks, involving widely distributed contaminated food products, than ever before (17).

Hypothesis

The purpose of this study was to group PFGE patterns of *Campylobacter* by animal exposure and geographic location in Utah. The hypothesis is that there is an association between PFGE patterns and specific animal exposure and/or geographic location in Utah.

CHAPTER II

MATERIALS AND METHODS

Sample Collection and Isolation

The Utah Health Code mandates in the Communicable Disease Rule R386-702, that all *Campylobacter* species are required to be submitted to the Unified State Laboratories: Public Health (USLPH). The USLPH has received thousands of *Campylobacter* isolates over the last decade, sent from clinical laboratories in Utah. Typically, presumptive colonies are submitted following isolation at the clinical laboratories. Occasionally primary stool samples are received at the USLPH, usually during outbreak situations.

Once a presumptive *Campylobacter* specimen is received, it is plated onto either a Campy CVA (Cefoperazone, Vancomycin, and Amphotericin B) agar with 5% sheep blood (Hardy) for stool, or TSA blood (Trypticase Soy Agar, Blood; Remel) plate for cultures already isolated and submitted by clinical laboratories. The plates are incubated overnight at 42ºC under microaerophilic conditions created in a 2.5 liter jar with 5% O_2, 10% CO_2 and 85% N_2 produced by a Oxoid CampyGen sachet (Hardy). The plates are examined at 24, 48, and 72 hours for characteristic growth. Colonies exhibiting typical *Campylobacter* morphology are

tested with biochemicals. *Campylobacter jejuni* is identified from a positive oxidase (Remel), catalase (Hardy) and hippurate (Hardy) reaction. All other *Campylobacter* species are differentiated by a negative hippurate reaction and then reported as *Campylobacter* species.

More than 2,000 *Campylobacter* samples have been submitted to the USLPH since 2002. The majority of these samples are from human patients, but some were isolated from water and raw milk during outbreaks. *Campylobacter* has also been detected in a sample collected directly from a cow. Although *Campylobacter* is most commonly isolated from stool, it has also been previously isolated from urine, blood, tissue, body fluid, and cervical swabs.

Demographics

A patient's specimen is identified at the local clinical laboratory, and a fax containing laboratory results of the presumptive diagnosis is sent to the state and local health department. Once the presumptive *Campylobacter* infection is reported to the local and state offices of epidemiology, an investigation begins. Public health investigators, typically nurses or epidemiologists from the local health departments, contact the patients by phone and collect a 72-hour food history. The Investigation Case Report Form is filled out that includes food and travel history and exposures to water, the outdoors, and animals. The information is then entered into Trisano, a public health database (or databases that preceded Trisano's implementation). From this, information is exported to an

Excel file where animal exposure and county of residence demographics are entered into the PFGE software, BioNumerics.

Pulsed-Field Gel Electrophoresis (PFGE)

PFGE is performed following the Ribot et al. version with slight modifications that are laboratory specific (36).

Plug preparation. A polyester-fiber or cotton swab that has been moistened with sterile phosphate-buffered saline (PBS: 0.01 M; pH 7.2-7.4; 0.85% NaCl; Sigma) is used to remove colonies from the agar plate containing fresh *Campylobacter* growth. The swab is used to suspend the cells in 2 to 3 ml of PBS in labeled 14 ml Falcon 2057 tubes (BD). A cell concentration of 0.52-0.64 OD is needed, determined by a Dade Microscan Turbidity Meter. Next, 400 µl of the adjusted cell suspensions are transferred to labeled 1.5 ml microcentrifuge tubes containing 20 µl of Proteinase K (20 mg/ml stock; Bioline). Then, 400 µl of the melted 1% SeaKem Gold agarose (Lonza) in TE buffer (10 mM Tris: 1 mM EDTA, pH 8.0) is added to the cell suspension and mixed by pipetting up and down two times and immediately dispensed into the appropriate wells of the disposable plug mold (BIO-RAD). The plugs are kept at room temperature for 10-15 minutes or at 4ºC for 5 minutes to set up.

Lysis of cells in agarose plugs. Once the plugs have cooled and solidified, they are added to their respectively labeled 50 ml polypropylene screw-cap tubes containing 5 ml cell lysis buffer (50 mM Tris:50 mM EDTA, pH 8.0 + 1% Sarcosyl) and 25 µl proteinase K stock solution. Lysis occurs in a 54ºC shaking water bath

for at least 15 minutes with constant and vigorous agitation (190 rpm). The water level in the water bath must remain above the lysis buffer in the tubes throughout the procedure.

Washes. Sterile reagent grade water and TE Buffer (10mM Tris:1 mM EDTA, pH 8.0) are preheated in a 56ºC water bath. To begin washings, the lysis buffer is poured off, while keeping the plugs in the tubes using screen caps (BIO-RAD). Each plug is washed six times total, twice with 10 ml of the preheated sterile reagent grade water and four times with 10 ml of the preheated sterile TE buffer. Each wash is done in the 54ºC shaking water bath, shaking vigorously, for at least 10 minutes. After the last wash, the plugs are stored in labeled 5 ml Falcon 2054 tubes (BD) containing 3 ml sterile TE buffer and stored at 4ºC.

Restriction digestion. Approximately 2 mm-wide slices are cut from each *Campylobacter* plug using a single-edge razor blade and transferred to a labeled 1.5 ml microcentrifuge tube containing 200 µl of the restriction enzyme mixture containing 40 U of *SmaI* (Roche/New England Biolabs). The plug slices are allowed to restrict for at least 4 hours at 25ºC. After restriction, the enzyme mixture is removed and replaced with 200 µl of 0.5X TBE (10X TBE contains 0.89 M Tris borate and 0.02 M EDTA, pH 8.3; Sigma) and allowed to stand at room temperature for 5 minutes. The restricted plug slices are loaded on a comb and a 1% SeaKem Gold Agarose in 0.5X TBE gel can be poured into the gel form. After 30 minutes, the comb and the frame of the gel form are removed, keeping the casting platform with the gel. The gel and platform are placed inside

the black gel frame in the electrophoresis chamber and the chamber's cover is closed.

Electrophoresis conditions. Electrophoresis is performed using a CHEF Mapper system (BIO-RAD). Gels are run with an initial switch time of 6.76 seconds increasing linearly to reach a final switch time of 35.38 seconds. Gels are subjected to electrophoresis for 19 hours at 6 V/cm in 0.5X TBE running buffer at 14ºC. Gels are stained with ethidium bromide (AMRESCO) and destained in water. Pattern visualization is obtained under UV transillumination aided by a Gel Doc XR gel analysis system (BIO-RAD).

Computer analysis of PFGE patterns. The PFGE patterns are analyzed using BioNumerics version 5.10 (Applied Maths). The TIFF images captured are analyzed in BioNumerics using the Dice correlation coefficient, with 1.5% optimization and 1.5% position tolerance, and dendograms, constructed using the UPGMA (unweighted pair group mathematical average) clustering algorithm with average linkages.

CHAPTER III

RESULTS

Of the more than 2,000 *Campylobacter* samples that have been collected at the USLPH since the beginning of 2002, 1,728 samples have yielded analyzable PFGE patterns. Among these analyzable patterns, 540 PFGE patterns have been distinguished, 381 of which are unique among Utah's database. This leaves only 159 patterns that have occurred more than once in Utah PFGE patient history, exemplifying the unusual diversity among *Campylobacter's* genome. Demographic information, including the patient's county of residence and animal exposures, provided by epidemiologists resulting from Investigation Case Report Forms, was imported into BioNumerics. Among the 1,728 analyzable patient patterns, 1,538 of these contain county of residence data and 718 include animal exposure data.

Utah is comprised of 29 counties. The USLPH has received patient samples from all but two counties in the last 10 years (Table 1). As expected, sample amounts reflect population size. According to the United States Census Bureau in 2009, Utah's population includes 2,784,572 people. Utah is the 11th largest state in the union in size but ranked 34th by population according to the

2009 census data and information provided by Utah's government website. With the majority of Utahans living along the Wasatch Front, a large proportion of land mass remains untouched by civilization. Outside the Salt Lake Valley, much of civilization is considered to be fairly rural. The extremes in population density seen across Utah caused the Utah Association of Counties to create six county classes based on population size to be identified (Figure 1). This largely rural lifestyle also allows Utahans to be exposed to a diverse group of animals.

Animal Exposure Analysis

The animal exposure data collected was organized into 10 groups based on the animals' physiological differences and sample amounts (Table 2). Campylobacter samples that include animal exposure data were analyzed two separate ways using BioNumerics. First, animal exposures were analyzed using BioNumerics' cluster tools by dividing the patterns into the ten different animal groups (Table 2). The total pattern amounts in each animal group were then calculated and each group's pattern representation exhibited (Figure 2). Human patterns with reported exposure to dogs represent almost half and reported cat exposure represents almost a quarter of the total reported animal exposures.

Next, all 10 of the animal groups were separated and analyzed independently in BioNumerics. In each animal group, relatedness was determined by dividing the total number of different patterns by the total number of samples in that animal group (Table 3). A higher pattern variation reflects a more diverse genetic makeup of the Campylobacters involved in the specific

animal group. Notably, only the canine and feline groups have less than 50% pattern diversity, exhibiting a greater majority of common patterns than the other groups.

Samples were also divided into groups of common patterns. Among these groups of patterns, only those with four or more samples containing animal exposure data were selected. This yielded 43 separate pattern clusters to analyze. A table was created outlining each of these 43 patterns and the percentage makeup of each animal group among that specific pattern (Table 4). In only looking at common patterns, some animal exposure data are lost. The animal exposure data representing common patterns are exhibited in Figure 3. The patterns that included reported animal exposure data that was lost when only looking at common PFGE patterns is visualized (Figure 4). This shows the loss of reported animal exposure data among common patterns was proportionate across all animal groups.

County of Residence Analysis

Campylobacter samples that include county of residence data were also analyzed two separate ways using BioNumerics. First, the patterns were organized using BioNumerics' cluster tools by dividing the patterns into the 27 different counties of residence, and the total pattern amounts in each county were then calculated (Table 1). In each county, relatedness was determined by dividing the total number of different patterns by the total number of samples (Table 5 and Figure 5). Again, a higher pattern variation reflects a more diverse

genetic makeup of the Campylobacters involved in the counties of origin. Notably, only Cache, Salt Lake, and Utah counties show a pattern variation of less than 50%. All but one of the counties with less than 10 total samples showed 100% variation with no repeating patterns.

Next, samples were divided into groups of common patterns. Among these groups of patterns, only those with four or more samples containing county of residence data where at least three samples originated from the same county were selected. This yielded 42 separate pattern clusters to analyze. A table was created outlining each of these 42 patterns and the percentage makeup of each county among that specific pattern (see Table 6).

These data enable common patterns to be broken down by county. The percentage of which each county contains the common pattern was calculated and outliers noted, seen highlighted in yellow (Table 6). These patterns appear to be more common in those counties. Notably, pattern UTDBDS16.065, UTDBDS16.134, UTDBDS16.214, and UTDBDS16.294 are most often found in Cache County. Patterns UTDBDS16.070, UTDBDS16.095, UTDBDS16.390, and UTDBDS16.600 are common in Utah County. Weber County is overwhelmingly the location common to pattern UTDBDS16.164 while Salt Lake County has several common patterns contained within its borders. The overwhelming majority of common patterns found most often in Salt Lake County are not surprising. Over 38% of Utahns live in Salt Lake County and are infected by Campylobacter much more compared to counties with smaller populations. With

that in mind, the smaller counties with elevated percentages of common patterns may be even more important.

Animal and County Analysis

The *Campylobacter* samples containing animal and county of residence information were then analyzed together. This information may help elucidate further what patterns are common in what animals in which counties (Table 7). Not surprisingly, Salt Lake County emerges again as the most common county with reported animal exposure among those with a *Campylobacter* infection. Interestingly though, cattle were most often reported as an exposure in Cache County, while felines and vermin were most represented in Utah County. This could implicate cats and vermin as sources for *Campylobacter* infections in Utah County. Exposure to cattle in Cache County could also possibly be a source for developing *Campylobacteriosis*.

Statistical Analysis

Pearson's correlation coefficient was the test statistic used to determine an association between common PFGE patterns and the exposure to different animal groups. Using the data found in Table 4, PFGE common patterns and their representation of reported animal exposure by groups were calculated using the two-tailed t-test for correlation. For each common pattern identified, the animal groups reported with that pattern were used. Forty-three common PFGE patterns (n = 43) were identified with representative reported animal exposures

resulting in 41 degrees of freedom. The critical value of 0.3932 was determined by the 41 degrees of freedom of a two-tailed t-test with a p-value of 0.01 (Table 8).

Pearson's correlation coefficient was also the test statistic used to determine the correlation between common PFGE patterns and the geographic location in Utah. Using the data found in Table 6, PFGE common patterns and representative Utah county locations were calculated using the two-tailed t-test for correlation. For each common pattern identified, the county reported with that pattern was used. Forty-two common PFGE patterns (n = 42) were identified with representative reported animal exposures resulting in 40 degrees of freedom. The critical value of 0.3932 was determined by the 40 degrees of freedom of a two-tailed t-test with a p-value set at 0.01 (Table 9).

Table 1.

Utah Counties, Populations, and PFGE Cases

County	Population	% of Total Population	PFGE Cases	% of Total Cases
Beaver	6,428	0.25%	11	0.72%
Box Elder	45,987	1.76%	31	2.02%
Cache	105,671	4.04%	206	13.40%
Carbon	19,504	0.75%	2	0.13%
Daggett	949	0.04%	0	0.00%
Davis	286,547	10.96%	75	4.88%
Duchesne	15,585	0.60%	2	0.13%
Emery	10,438	0.40%	0	0.00%
Garfield	4,772	0.18%	4	0.26%
Grand	9,024	0.35%	4	0.26%
Iron	43,424	1.66%	28	1.82%
Juab	9,315	0.36%	15	0.98%
Kane	6,294	0.24%	5	0.33%
Millard	13,230	0.51%	28	1.82%
Morgan	8,888	0.34%	2	0.13%
Piute	1,373	0.05%	2	0.13%
Rich	2,121	0.08%	2	0.13%
Salt Lake	996,374	38.10%	527	34.29%
San Juan	14,647	0.56%	1	0.07%
Sanpete	25,799	0.99%	45	2.93%
Sevier	19,984	0.76%	23	1.50%
Summit	36,871	1.41%	33	2.15%
Tooele	54,375	2.08%	13	0.85%
Uintah	27,747	1.06%	7	0.46%
Utah	475,425	18.18%	283	18.41%
Wasatch	21,053	0.81%	25	1.63%
Washington	134,899	5.16%	77	5.01%
Wayne	2,535	0.10%	6	0.39%
Weber	215,870	8.25%	80	5.20%
Total	2,615,129	100.00%	1,537	100.00%

Based on the Utah Association of Counties 2006 Population

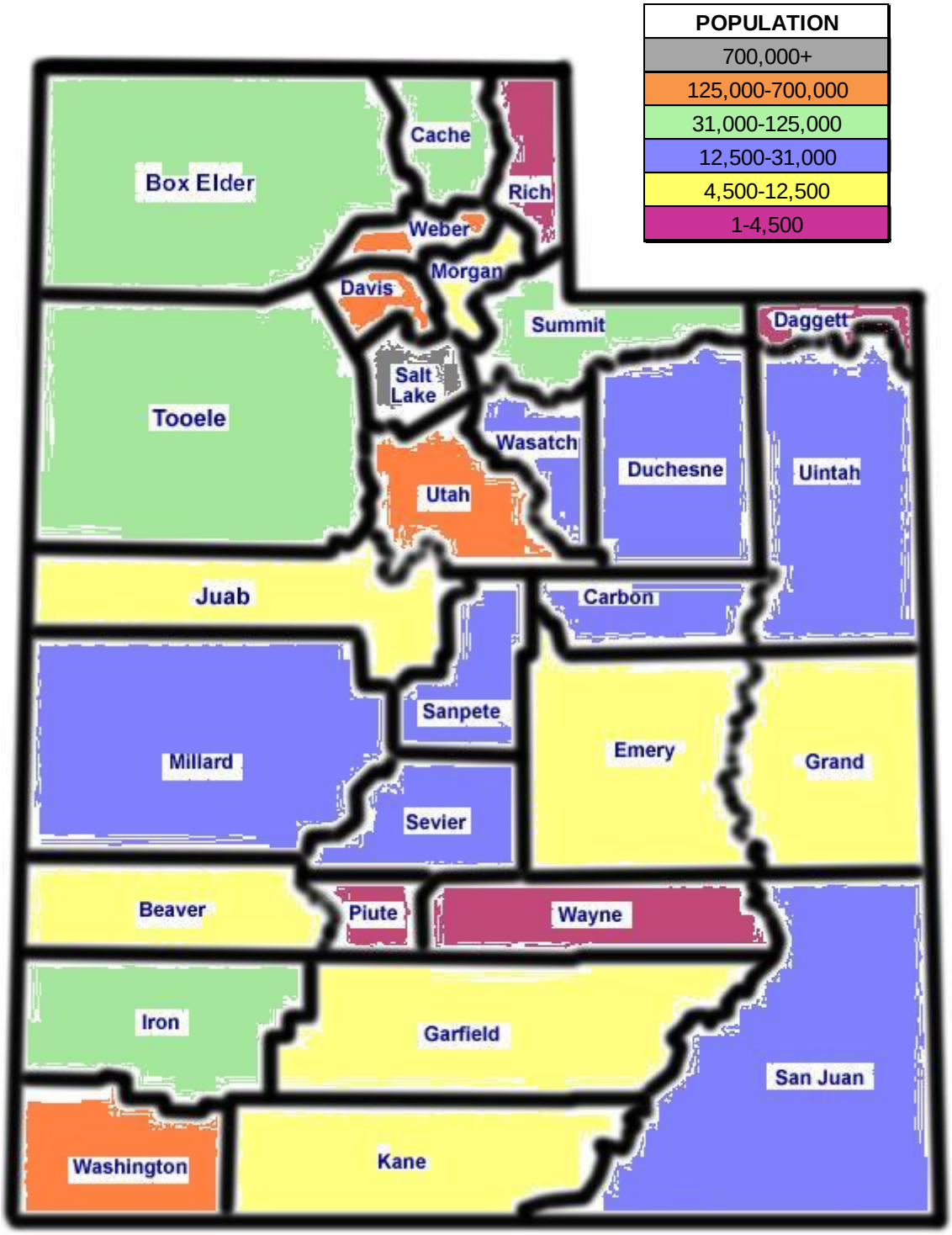

Figure 1.

Utah County Map Separated by Population

Table 2.

Animal Grouping and Human PFGE Patterns Associated

Group	Included Animals	PFGE Cases
Birds (2.95%)	Bird (23), Cockatiel (1), Parakeet (3), Pigeon (2), Parrot (2), Lovebird (1)	32
Canine (47.79%)	Dog (480), Puppy (53)	518
Cattle (5.63%)	Cattle (3), Cow (45), Calf (15), Calves (4), Bull (1), Dairy (2)	61
Cold-Blooded Vertebrates (1.48%)	Fish (6), Frog (3), Goldfish (3), Snake (2), Lizard (1), Gecko (1)	16
Feline (24.82%)	Cat (254), Kitten (23)	269
Poultry (6.27%)	Chicken (50), Duck (14), Quail (1), Chicks (3), Turkey (5), Geese (1), Fowl (1), Pheasants (1)	68
Random Animals (0.28%)	Scorpion (1), Monkeys (1), Shark (1), Sting Ray (1)	3
Ruminants (Noncattle) (2.58%)	Camel (1), Deer (2), Goat (13), Lamb (3), Sheep (9), Llama (1), Moose (1), Pigmy Goat (1)	28
Simple Stomach (5.35%)	Donkey (2), Horse (44); Pig (11), Hog (1)	55
Vermin (2.95%)	Mice (4), Gerbil (2), Hamster (6), Mink (1), Ferret (1), Rat (2), Guinea Pig (3), Skunk (1), Racoon (1), Rabbit (13), Bunnies (1)	33
	Total	1083

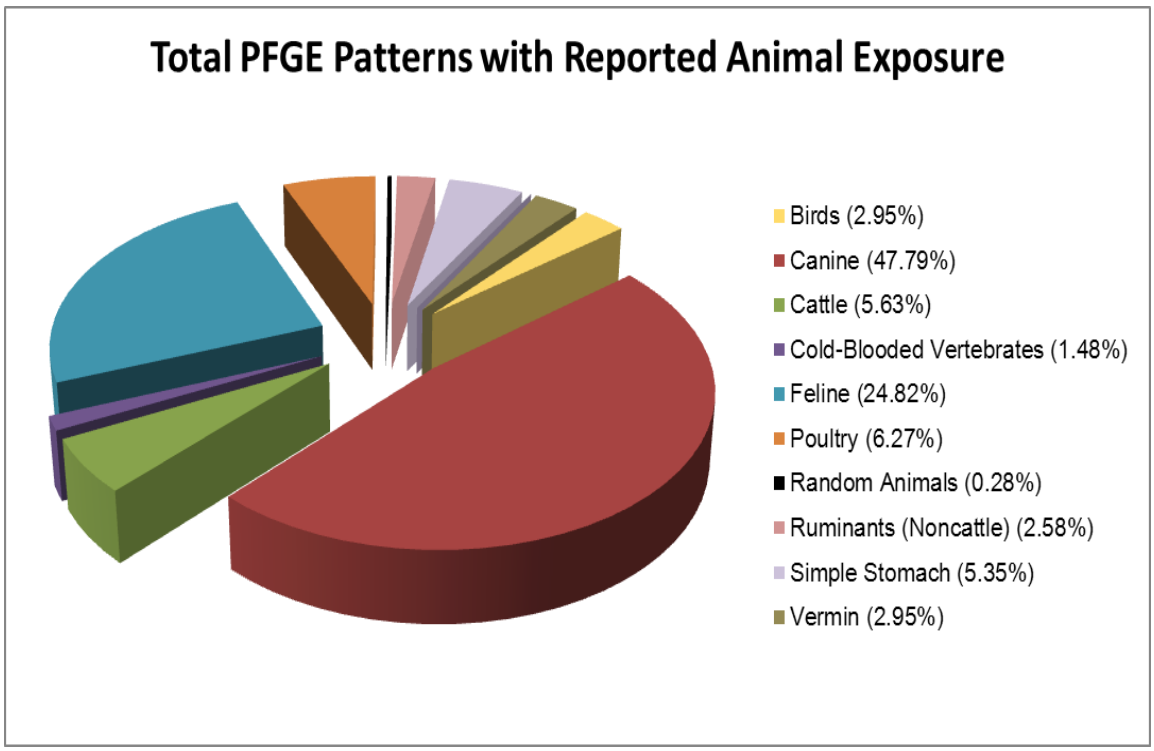

Figure 2.

Animal Groups Representation

Table 3.

PFGE Pattern Variation among Animal Groups

Animal Group	Patterns	Total Samples	Pattern Variation
Birds	25	32	78.13%
Canine	210	518	40.54%
Cattle	36	61	59.02%
Cold-Blooded Vertebrates	11	16	68.75%
Feline	129	269	47.96%
Poultry	46	68	67.65%
Random Animals	3	3	100%
Ruminants (Noncattle)	22	28	78.57%
Simple Stomach	39	55	70.91%
Vermin	23	33	69.70%
Total	544	1083	

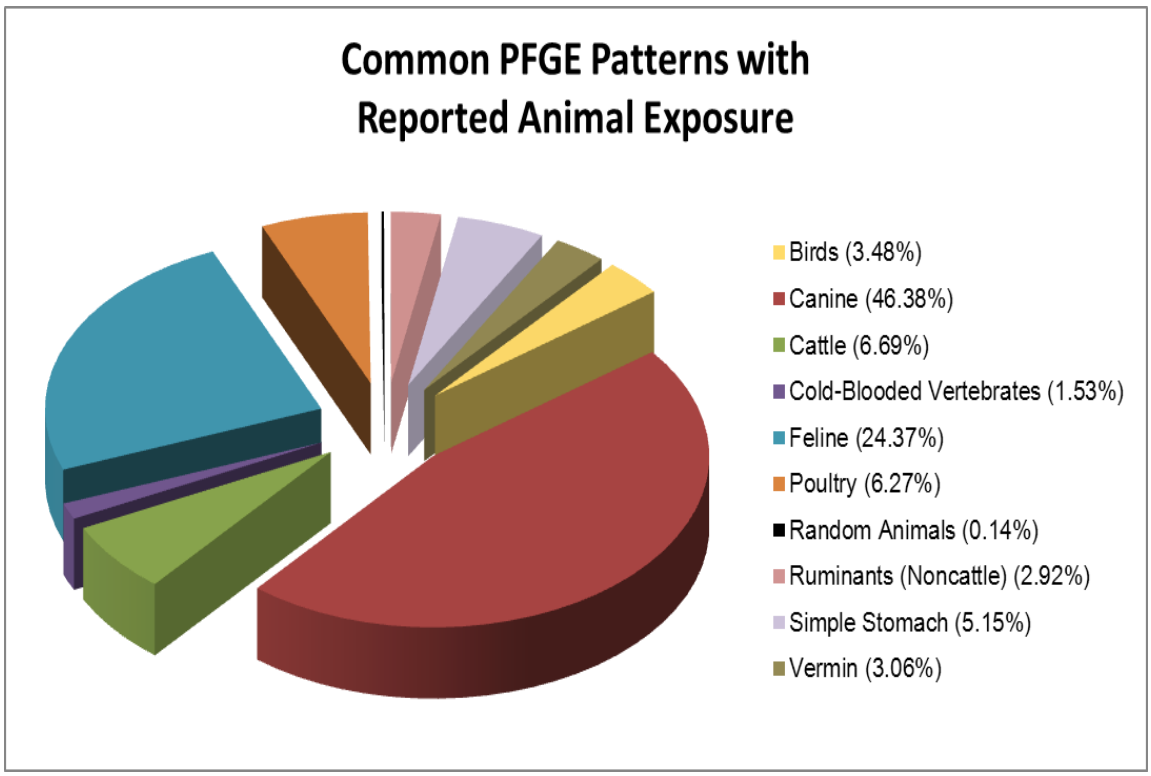

Figure 3.

Animal Groups Representation among Common PFGE Patterns

Table 4.

Reported Animal Exposures among Common PFGE Patterns (n = 718)

		Bird		Canine		Cattle		Cold-blooded		Feline		Poultry		Random		Ruminants		Simple Stomach		Vermin	
UT Smal pattern	Pattern Amount	Pattern	% of Pattern	Pattern	% of Pattern	Pattern	% of Pattern	Pattern	% of Pattern	Pattern	% of Pattern	Pattern	% of Pattern	Pattern	% of Pattern	Pattern	% of Pattern	Pattern	% of Pattern	Pattern	% of Pattern
UTDBDS16.009	101	2	1.98%	19	18.81%	1	0.99%	3	2.97%	14	13.86%	3	2.97%	0	0.00%	1	0.99%	3	2.97%	3	2.97%
UTDBDS16.130	99	1	1.01%	31	31.31%	6	6.06%	0	0.00%	15	15.15%	4	4.04%	0	0.00%	1	1.01%	5	5.05%	2	2.02%
UTDBDS16.074	72	1	1.39%	27	37.50%	5	6.94%	0	0.00%	12	16.67%	1	1.39%	0	0.00%	2	2.78%	6	8.33%	2	2.78%
UTDBDS16.011	71	2	2.82%	28	39.44%	1	1.41%	1	1.41%	18	25.35%	2	2.82%	0	0.00%	2	2.82%	0	0.00%	4	5.63%
UTDBDS16.103	66	1	1.52%	25	37.88%	2	3.03%	2	3.03%	17	25.76%	1	1.52%	1	1.52%	4	6.06%	3	4.55%	1	1.52%
UTDBDS16.286	45	2	4.44%	10	22.22%	1	2.22%	0	0.00%	6	13.33%	2	4.44%	0	0.00%	1	2.22%	1	2.22%	2	4.44%
UTDBDS16.143	33	3	9.09%	13	39.39%	0	0.00%	0	0.00%	10	30.30%	1	3.03%	0	0.00%	1	3.03%	0	0.00%	0	0.00%
UTDBDS16.222	30	0	0.00%	6	20.00%	1	3.33%	0	0.00%	3	10.00%	2	6.67%	0	0.00%	1	3.33%	1	3.33%	1	3.33%
UTDBDS16.353	29	2	6.90%	15	51.72%	0	0.00%	0	0.00%	7	24.14%	4	13.79%	0	0.00%	2	6.90%	1	3.45%	1	3.45%
UTDBDS16.134	28	1	3.57%	11	39.29%	3	10.71%	0	0.00%	5	17.86%	1	3.57%	0	0.00%	0	0.00%	1	3.57%	0	0.00%
UTDBDS16.390	28	2	7.14%	4	14.29%	0	0.00%	2	7.14%	5	17.86%	1	3.57%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.177	27	0	0.00%	10	37.04%	0	0.00%	0	0.00%	5	18.52%	1	3.70%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.199	25	0	0.00%	6	24.00%	2	8.00%	1	4.00%	6	24.00%	1	4.00%	0	0.00%	1	4.00%	1	4.00%	0	0.00%
UTDBDS16.098	23	0	0.00%	6	26.09%	4	17.39%	1	0.00%	2	8.70%	2	8.70%	0	0.00%	1	4.35%	0	0.00%	0	0.00%
UTDBDS16.183	22	0	0.00%	7	31.82%	5	22.73%	1	4.55%	3	13.64%	0	0.00%	0	0.00%	1	4.55%	1	4.55%	1	4.55%
UTDBDS16.600	22	0	0.00%	8	36.36%	1	4.55%	0	0.00%	1	4.55%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.235	21	0	0.00%	11	52.38%	0	0.00%	0	0.00%	0	0.00%	1	4.76%	0	0.00%	0	0.00%	0	0.00%	1	4.76%
UTDBDS16.159	19	0	0.00%	6	31.58%	1	5.26%	0	0.00%	3	15.79%	5	26.32%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.558	18	1	5.56%	8	44.44%	0	0.00%	0	0.00%	6	33.33%	2	11.11%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.155	16	1	6.25%	5	31.25%	1	6.25%	0	0.00%	2	12.50%	0	0.00%	0	0.00%	0	0.00%	1	6.25%	0	0.00%
Total	988	25		331		48		12		174		45		1		21		37		22	

Table 4 – Continued

UT Smal pattern	Pattern Amount	Bird		Canine		Cattle		Cold-blooded		Feline		Poultry		Random		Ruminants		Simple Stomach		Vermin	
		Pattern	% of Pattern	Pattern	% of Pattern	Pattern	% of Pattern	Pattern	% of Pattern	Pattern	% of Pattern	Pattern	% of Pattern	Pattern	% of Pattern	Pattern	% of Pattern	Pattern	% of Pattern	Pattern	% of Pattern
UTDBDS16.499	15	0	0.00%	3	20.00%	0	0.00%	0	0.00%	2	13.33%	1	6.67%	0	0.00%	1	6.67%	0	0.00%	0	0.00%
UTDBDS16.067	14	1	7.14%	4	28.57%	1	7.14%	0	0.00%	2	14.29%	1	7.14%	0	0.00%	1	7.14%	2	14.29%	0	0.00%
UTDBDS16.484	14	0	0.00%	6	42.86%	0	0.00%	0	0.00%	2	14.29%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.095	12	0	0.00%	4	33.33%	2	16.67%	0	0.00%	1	8.33%	1	8.33%	0	0.00%	0	0.00%	1	8.33%	0	0.00%
UTDBDS16.200	11	0	0.00%	3	27.27%	0	0.00%	0	0.00%	3	27.27%	1	9.09%	0	0.00%	0	0.00%	1	9.09%	0	0.00%
UTDBDS16.280	11	0	0.00%	3	27.27%	0	0.00%	0	0.00%	1	9.09%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.294	11	0	0.00%	7	63.64%	1	9.09%	0	0.00%	3	27.27%	0	0.00%	0	0.00%	0	0.00%	1	9.09%	2	18.18%
UTDBDS16.412	10	0	0.00%	6	60.00%	0	0.00%	0	0.00%	3	30.00%	0	0.00%	0	0.00%	0	0.00%	2	20.00%	0	0.00%
UTDBDS16.189	9	0	0.00%	3	33.33%	1	11.11%	0	0.00%	1	11.11%	0	0.00%	0	0.00%	0	0.00%	1	11.11%	0	0.00%
UTDBDS16.205	9	1	11.11%	1	11.11%	1	11.11%	0	0.00%	1	11.11%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.070	8	1	12.50%	1	12.50%	0	0.00%	0	0.00%	2	25.00%	3	37.50%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.075	7	0	0.00%	1	14.29%	2	28.57%	0	0.00%	1	14.29%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.080	7	0	0.00%	2	28.57%	0	0.00%	0	0.00%	0	0.00%	1	14.29%	0	0.00%	0	0.00%	0	0.00%	1	14.29%
UTDBDS16.164	7	0	0.00%	3	42.86%	0	0.00%	0	0.00%	1	14.29%	0	0.00%	0	0.00%	0	0.00%	1	14.29%	0	0.00%
UTDBDS16.221	7	1	14.29%	4	57.14%	2	28.57%	0	0.00%	2	28.57%	2	28.57%	0	0.00%	1	14.29%	2	28.57%	0	0.00%
UTDBDS16.581	7	0	0.00%	4	57.14%	3	42.86%	0	0.00%	2	28.57%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.085	5	0	0.00%	4	80.00%	0	0.00%	0	0.00%	1	20.00%	1	20.00%	0	0.00%	0	0.00%	1	20.00%	0	0.00%
UTDBDS16.109	5	0	0.00%	2	40.00%	0	0.00%	0	0.00%	2	40.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.225	5	0	0.00%	2	40.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	1	20.00%
UTDBDS16.152	4	0	0.00%	3	75.00%	0	0.00%	0	0.00%	1	25.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.359	4	0	0.00%	3	75.00%	0	0.00%	0	0.00%	1	25.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.364	4	1	25.00%	3	75.00%	1	25.00%	1	25.00%	2	50.00%	0	0.00%	0	0.00%	0	0.00%	1	25.00%	0	0.00%
UTDBDS16.402	4	1	25.00%	3	75.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
Total	988	25		331		48		12		174		45		1		21		37		22	

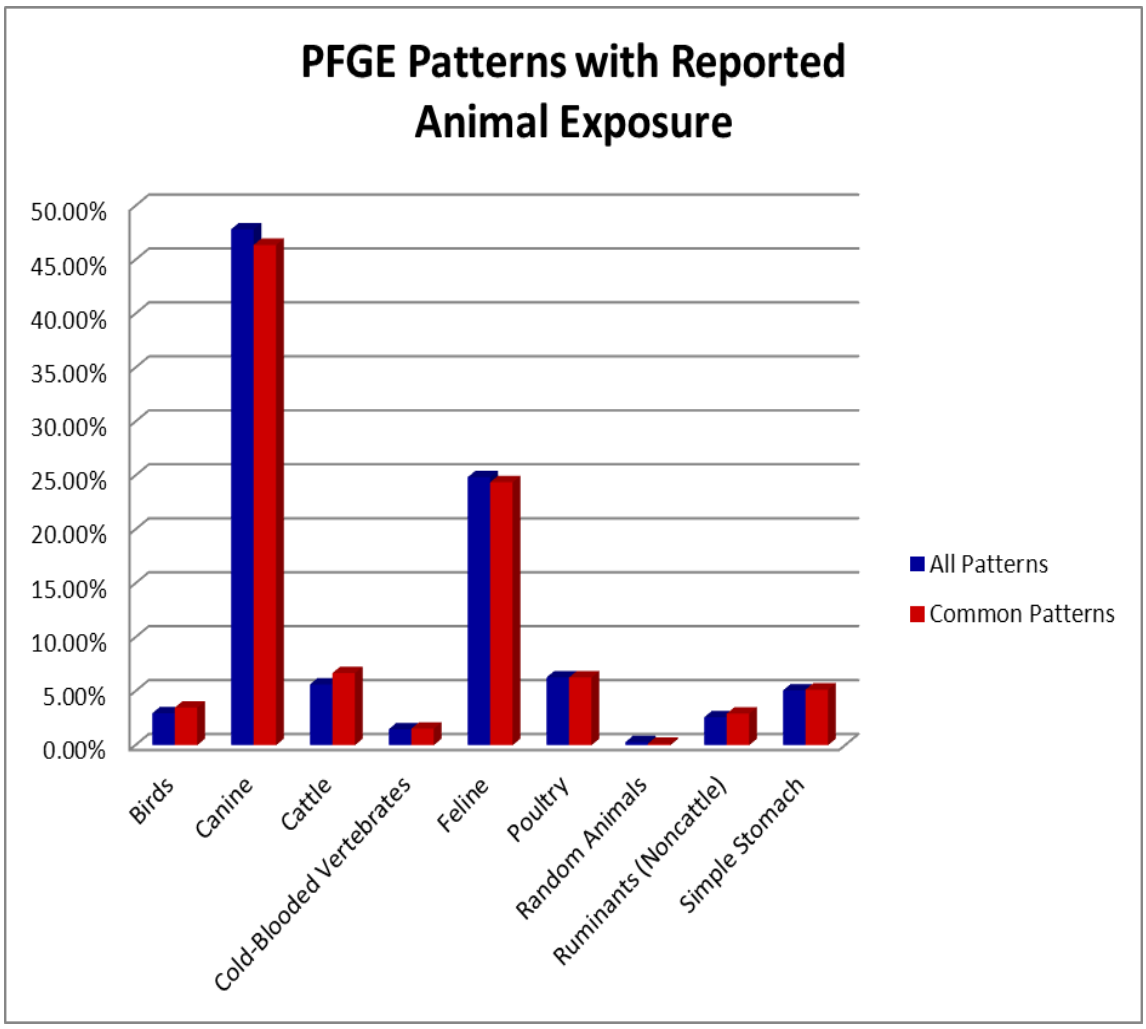

Figure 4.

Reported Animal Exposure (Total Samples vs. Common Pattern Representation)

Table 5.

PFGE Pattern Variation by County

County	Patterns	Total Samples	Pattern Variation
Beaver	10	11	90.91%
Box Elder	22	31	70.97%
Cache	93	206	45.15%
Carbon	2	2	100.00%
Davis	56	75	74.67%
Duchesne	2	2	100.00%
Garfield	4	4	100.00%
Grand	4	4	100.00%
Iron	24	28	85.71%
Juab	11	15	73.33%
Kane	4	5	80.00%
Millard	20	28	71.43%
Morgan	2	2	100.00%
Piute	2	2	100.00%
Rich	2	2	100.00%
Salt Lake	253	527	48.01%
San Juan	1	1	100.00%
Sanpete	37	45	82.22%
Sevier	15	23	65.22%
Summit	23	33	69.70%
Tooele	10	13	76.92%
Uintah	7	7	100.00%
Utah	136	283	48.06%
Wasatch	22	25	88.00%
Washington	49	77	63.64%
Wayne	6	6	100.00%
Weber	48	80	60.00%
Total	865	1537	

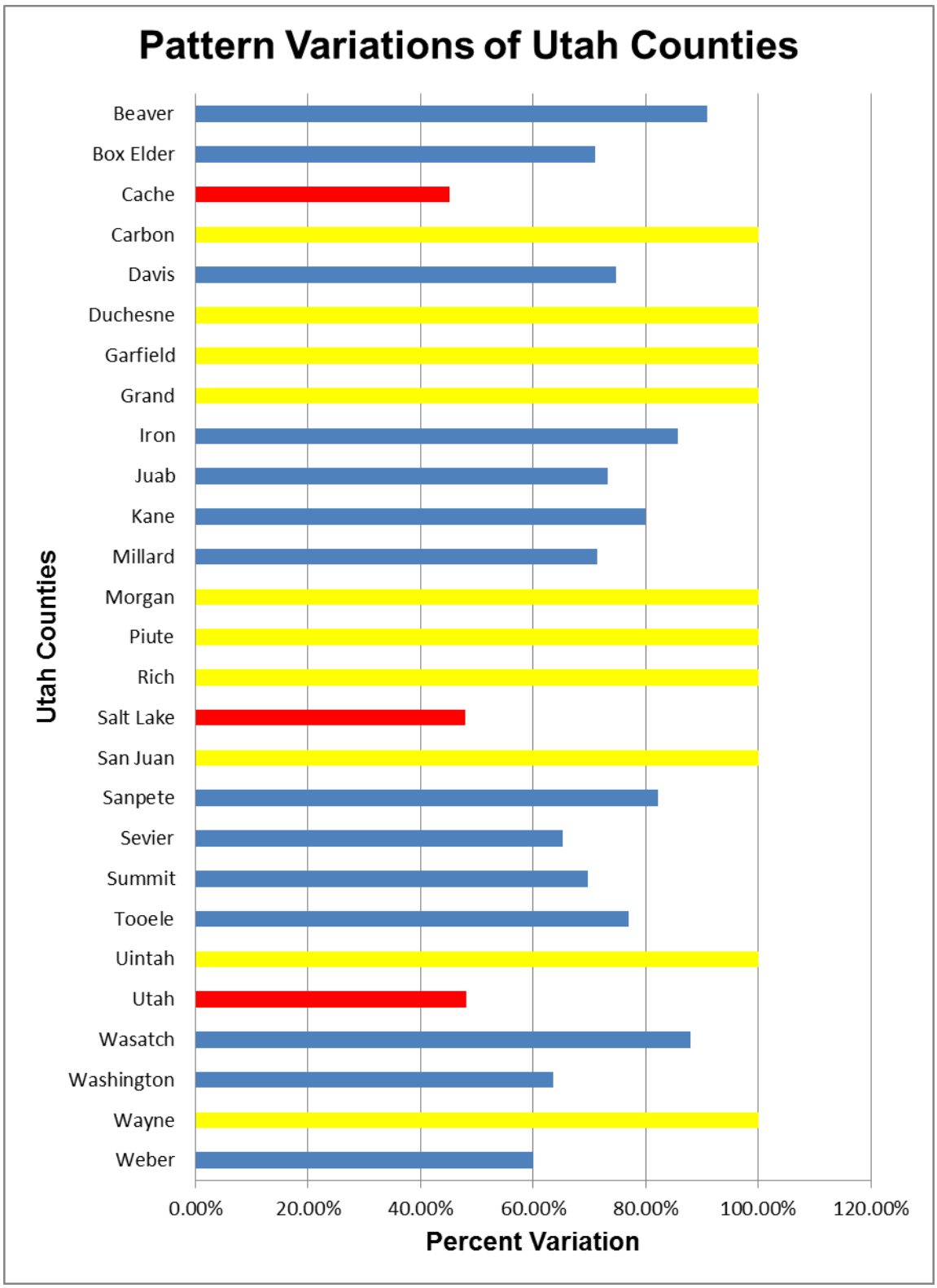

Figure 5.

Pattern Variations of Utah Counties

Table 6

Reported County of Residence among Common PFGE Patterns (n = 857)

UT Smal pattern	Pattern Amounts	Beaver Patterns	Beaver % of Patterns	Box Elder Patterns	Box Elder % of Patterns	Cache Patterns	Cache % of Patterns	Carbon Patterns	Carbon % of Patterns
UTDBDS16.009	88	0	0.00%	1	1.14%	13	14.77%	0	0.00%
UTDBDS16.011	60	0	0.00%	2	3.33%	5	8.33%	0	0.00%
UTDBDS16.065	6	0	0.00%	0	0.00%	5	83.33%	0	0.00%
UTDBDS16.067	12	1	8.33%	0	0.00%	2	16.67%	0	0.00%
UTDBDS16.070	8	0	0.00%	0	0.00%	1	12.50%	0	0.00%
UTDBDS16.074	64	2	3.13%	4	6.25%	17	26.56%	0	0.00%
UTDBDS16.080	7	0	0.00%	0	0.00%	2	28.57%	0	0.00%
UTDBDS16.095	10	0	0.00%	0	0.00%	1	10.00%	0	0.00%
UTDBDS16.098	19	0	0.00%	1	5.26%	7	36.84%	0	0.00%
UTDBDS16.103	59	1	1.69%	2	3.39%	7	11.86%	0	0.00%
UTDBDS16.109	5	1	20.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.110	4	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.130	89	0	0.00%	3	3.37%	14	15.73%	0	0.00%
UTDBDS16.134	21	0	0.00%	1	4.76%	9	42.86%	1	4.76%
UTDBDS16.143	28	1	3.57%	1	3.57%	5	17.86%	0	0.00%
UTDBDS16.155	13	0	0.00%	1	7.69%	3	23.08%	0	0.00%
UTDBDS16.159	17	1	5.88%	0	0.00%	2	11.76%	0	0.00%
UTDBDS16.163	5	0	0.00%	0	0.00%	1	20.00%	0	0.00%
UTDBDS16.164	7	0	0.00%	0	0.00%	1	14.29%	0	0.00%
UTDBDS16.167	8	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.183	18	0	0.00%	1	5.56%	3	16.67%	0	0.00%
UTDBDS16.199	22	0	0.00%	0	0.00%	6	27.27%	0	0.00%
UTDBDS16.200	11	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.205	9	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.214	7	0	0.00%	0	0.00%	3	42.86%	0	0.00%
UTDBDS16.222	23	0	0.00%	0	0.00%	5	21.74%	0	0.00%
UTDBDS16.225	5	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.232	5	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.234	8	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.235	21	0	0.00%	0	0.00%	1	4.76%	0	0.00%
UTDBDS16.262	4	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.271	7	0	0.00%	0	0.00%	1	14.29%	0	0.00%
UTDBDS16.280	10	0	0.00%	0	0.00%	1	10.00%	0	0.00%
UTDBDS16.286	36	0	0.00%	1	2.78%	6	16.67%	0	0.00%
UTDBDS16.294	11	0	0.00%	0	0.00%	3	27.27%	0	0.00%
UTDBDS16.353	29	0	0.00%	1	3.45%	4	13.79%	0	0.00%
UTDBDS16.390	28	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.412	10	0	0.00%	0	0.00%	2	20.00%	0	0.00%
UTDBDS16.484	11	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.499	15	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.558	16	0	0.00%	0	0.00%	1	6.25%	1	6.25%
UTDBDS16.600	21	0	0.00%	0	0.00%	0	0.00%	0	0.00%
Total	857	7		19		131		2	

Table 6 – Continued

UT Smal pattern	Pattern Amounts	Davis Patterns	Davis % of Patterns	Duchesne Patterns	Duchesne % of Patterns	Garfield Patterns	Garfield % of Patterns	Grand Patterns	Grand % of Patterns	Iron Patterns	Iron % of Patterns
UTDBDS16.009	88	4	4.55%	1	1.14%	0	0.00%	0	0.00%	2	2.27%
UTDBDS16.011	60	2	3.33%	0	0.00%	1	1.67%	0	0.00%	0	0.00%
UTDBDS16.065	6	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.067	12	1	8.33%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.070	8	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.074	64	3	4.69%	0	0.00%	0	0.00%	0	0.00%	1	1.56%
UTDBDS16.080	7	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.095	10	1	10.00%	0	0.00%	0	0.00%	0	0.00%	1	10.00%
UTDBDS16.098	19	0	0.00%	0	0.00%	0	0.00%	0	0.00%	1	5.26%
UTDBDS16.103	59	4	6.78%	0	0.00%	0	0.00%	0	0.00%	2	3.39%
UTDBDS16.109	5	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.110	4	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.130	89	3	3.37%	0	0.00%	1	1.12%	0	0.00%	1	1.12%
UTDBDS16.134	21	1	4.76%	0	0.00%	0	0.00%	0	0.00%	1	4.76%
UTDBDS16.143	28	0	0.00%	0	0.00%	1	3.57%	0	0.00%	1	3.57%
UTDBDS16.155	13	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.159	17	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.163	5	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.164	7	2	28.57%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.167	8	1	12.50%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.183	18	1	5.56%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.199	22	1	4.55%	0	0.00%	1	4.55%	0	0.00%	0	0.00%
UTDBDS16.200	11	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.205	9	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.214	7	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.222	23	0	0.00%	0	0.00%	0	0.00%	1	4.35%	0	0.00%
UTDBDS16.225	5	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.232	5	1	20.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.234	8	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.235	21	0	0.00%	1	4.76%	0	0.00%	0	0.00%	1	4.76%
UTDBDS16.262	4	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.271	7	1	14.29%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.280	10	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.286	36	2	5.56%	0	0.00%	0	0.00%	0	0.00%	1	2.78%
UTDBDS16.294	11	0	0.00%	0	0.00%	0	0.00%	0	0.00%	1	9.09%
UTDBDS16.353	29	5	17.24%	0	0.00%	0	0.00%	0	0.00%	1	3.45%
UTDBDS16.390	28	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.412	10	0	0.00%	0	0.00%	0	0.00%	0	0.00%	1	10.00%
UTDBDS16.484	11	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.499	15	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.558	16	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.600	21	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
Total	857	33		2		4		1		15	

Table 6 – Continued

UT Smal pattern	Pattern Amounts	Juab Patterns	Juab % of Patterns	Kane Patterns	Kane % of Patterns	Millard Patterns	Millard % of Patterns	Morgan Patterns	Piute Patterns	Piute % of Patterns
UTDBDS16.009	88	4	4.55%	0	0.00%	5	5.68%	0	0	0.00%
UTDBDS16.011	60	1	1.67%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.065	6	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.067	12	0	0.00%	0	0.00%	1	8.33%	0	0	0.00%
UTDBDS16.070	8	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.074	64	1	1.56%	0	0.00%	2	3.13%	0	1	1.56%
UTDBDS16.080	7	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.095	10	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.098	19	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.103	59	1	1.69%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.109	5	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.110	4	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.130	89	1	1.12%	2	2.25%	2	2.25%	0	0	0.00%
UTDBDS16.134	21	0	0.00%	0	0.00%	1	4.76%	0	0	0.00%
UTDBDS16.143	28	0	0.00%	0	0.00%	1	3.57%	0	0	0.00%
UTDBDS16.155	13	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.159	17	0	0.00%	0	0.00%	2	11.76%	0	0	0.00%
UTDBDS16.163	5	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.164	7	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.167	8	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.183	18	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.199	22	0	0.00%	0	0.00%	1	4.55%	0	0	0.00%
UTDBDS16.200	11	0	0.00%	0	0.00%	1	9.09%	0	0	0.00%
UTDBDS16.205	9	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.214	7	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.222	23	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.225	5	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.232	5	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.234	8	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.235	21	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.262	4	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.271	7	0	0.00%	0	0.00%	1	14.29%	0	0	0.00%
UTDBDS16.280	10	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.286	36	2	5.56%	0	0.00%	2	5.56%	0	0	0.00%
UTDBDS16.294	11	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.353	29	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.390	28	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.412	10	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.484	11	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.499	15	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.558	16	0	0.00%	0	0.00%	0	0.00%	0	0	0.00%
UTDBDS16.600	21	0		0	0.00%	0	0.00%	0	0	0.00%
Total	857	10		2		19		0	1	

Table 6 – Continued

UT Smal pattern	Pattern Amounts	Rich Patterns	Rich % of Patterns	Salt Lake Patterns	Salt Lake % of Patterns	San Juan Patterns	Sanpete Patterns	Sanpete % of Patterns	Sevier Patterns	Sevier % of Patterns
UTDBDS16.009	88	1	1.14%	21	23.86%	0	3	3.41%	3	3.41%
UTDBDS16.011	60	0	0.00%	22	36.67%	0	0	0.00%	5	8.33%
UTDBDS16.065	6	0	0.00%	1	16.67%	0	0	0.00%	0	0.00%
UTDBDS16.067	12	0	0.00%	3	25.00%	0	1	8.33%	0	0.00%
UTDBDS16.070	8	0	0.00%	3	37.50%	0	0	0.00%	0	0.00%
UTDBDS16.074	64	0	0.00%	11	17.19%	0	3	4.69%	2	3.13%
UTDBDS16.080	7	0	0.00%	3	42.86%	0	0	0.00%	0	0.00%
UTDBDS16.095	10	0	0.00%	0	0.00%	0	0	0.00%	0	0.00%
UTDBDS16.098	19	0	0.00%	3	15.79%	0	0	0.00%	0	0.00%
UTDBDS16.103	59	1	1.69%	22	37.29%	0	1	1.69%	1	1.69%
UTDBDS16.109	5	0	0.00%	3	60.00%	0	1	20.00%	0	0.00%
UTDBDS16.110	4	0	0.00%	3	75.00%	0	0	0.00%	0	0.00%
UTDBDS16.130	89	0	0.00%	29	32.58%	0	2	2.25%	1	1.12%
UTDBDS16.134	21	0	0.00%	3	14.29%	0	0	0.00%	0	0.00%
UTDBDS16.143	28	0	0.00%	5	17.86%	0	2	7.14%	0	0.00%
UTDBDS16.155	13	0	0.00%	3	23.08%	0	0	0.00%	0	0.00%
UTDBDS16.159	17	0	0.00%	4	23.53%	0	0	0.00%	0	0.00%
UTDBDS16.163	5	0	0.00%	3	60.00%	0	0	0.00%	0	0.00%
UTDBDS16.164	7	0	0.00%	1	14.29%	0	0	0.00%	0	0.00%
UTDBDS16.167	8	0	0.00%	5	62.50%	0	0	0.00%	0	0.00%
UTDBDS16.183	18	0	0.00%	5	27.78%	0	2	11.11%	0	0.00%
UTDBDS16.199	22	0	0.00%	8	36.36%	0	0	0.00%	1	4.55%
UTDBDS16.200	11	0	0.00%	6	54.55%	0	0	0.00%	1	9.09%
UTDBDS16.205	9	0	0.00%	5	55.56%	0	0	0.00%	0	0.00%
UTDBDS16.214	7	0	0.00%	2	28.57%	0	0	0.00%	0	0.00%
UTDBDS16.222	23	0	0.00%	3	13.04%	0	2	8.70%	0	0.00%
UTDBDS16.225	5	0	0.00%	4	80.00%	0	0	0.00%	0	0.00%
UTDBDS16.232	5	0	0.00%	3	60.00%	0	0	0.00%	0	0.00%
UTDBDS16.234	8	0	0.00%	3	37.50%	0	0	0.00%	0	0.00%
UTDBDS16.235	21	0	0.00%	13	61.90%	0	1	4.76%	0	0.00%
UTDBDS16.262	4	0	0.00%	3	75.00%	0	0	0.00%	0	0.00%
UTDBDS16.271	7	0	0.00%	3	42.86%	0	0	0.00%	0	0.00%
UTDBDS16.280	10	0	0.00%	5	50.00%	0	0	0.00%	0	0.00%
UTDBDS16.286	36	0	0.00%	6	16.67%	0	0	0.00%	0	0.00%
UTDBDS16.294	11	0	0.00%	1	9.09%	0	1	9.09%	0	0.00%
UTDBDS16.353	29	0	0.00%	8	27.59%	0	0	0.00%	1	3.45%
UTDBDS16.390	28	0	0.00%	7	25.00%	0	0	0.00%	0	0.00%
UTDBDS16.412	10	0	0.00%	4	40.00%	0	0	0.00%	0	0.00%
UTDBDS16.484	11	0	0.00%	7	63.64%	0	0	0.00%	0	0.00%
UTDBDS16.499	15	0	0.00%	11	73.33%	0	0	0.00%	0	0.00%
UTDBDS16.558	16	0	0.00%	10	62.50%	0	0	0.00%	0	0.00%
UTDBDS16.600	21	0	0.00%	3	14.29%	0	0	0.00%	0	0.00%
Total	857	2		268		0	19		15	

Table 6 – Continued

UT Smal pattern	Pattern Amounts	Summit Patterns	Summit % of Patterns	Tooele Patterns	Tooele % of Patterns	Uintah Patterns	Uintah % of Patterns	Utah Patterns	Utah % of Patterns
UTDBDS16.009	88	3	3.41%	0	0.00%	0	0.00%	14	15.91%
UTDBDS16.011	60	2	3.33%	1	1.67%	0	0.00%	12	20.00%
UTDBDS16.065	6	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.067	12	0	0.00%	0	0.00%	0	0.00%	3	25.00%
UTDBDS16.070	8	0	0.00%	0	0.00%	0	0.00%	3	37.50%
UTDBDS16.074	64	0	0.00%	0	0.00%	1	1.56%	12	18.75%
UTDBDS16.080	7	0	0.00%	0	0.00%	0	0.00%	1	14.29%
UTDBDS16.095	10	0	0.00%	1	10.00%	1	10.00%	4	40.00%
UTDBDS16.098	19	0	0.00%	0	0.00%	0	0.00%	3	15.79%
UTDBDS16.103	59	0	0.00%	1	1.69%	1	1.69%	8	13.56%
UTDBDS16.109	5	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.110	4	0	0.00%	0	0.00%	0	0.00%	1	25.00%
UTDBDS16.130	89	0	0.00%	2	2.25%	0	0.00%	9	10.11%
UTDBDS16.134	21	0	0.00%	0	0.00%	0	0.00%	2	9.52%
UTDBDS16.143	28	2	7.14%	0	0.00%	0	0.00%	3	10.71%
UTDBDS16.155	13	1	7.69%	0	0.00%	0	0.00%	3	23.08%
UTDBDS16.159	17	0	0.00%	0	0.00%	0	0.00%	4	23.53%
UTDBDS16.163	5	0	0.00%	0	0.00%	0	0.00%	1	20.00%
UTDBDS16.164	7	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.167	8	0	0.00%	0	0.00%	0	0.00%	2	25.00%
UTDBDS16.183	18	0	0.00%	0	0.00%	0	0.00%	4	22.22%
UTDBDS16.199	22	0	0.00%	1	4.55%	0	0.00%	2	9.09%
UTDBDS16.200	11	1	9.09%	0	0.00%	0	0.00%	1	9.09%
UTDBDS16.205	9	1	11.11%	0	0.00%	0	0.00%	2	22.22%
UTDBDS16.214	7	0	0.00%	0	0.00%	0	0.00%	1	14.29%
UTDBDS16.222	23	0	0.00%	0	0.00%	1	4.35%	6	26.09%
UTDBDS16.225	5	0	0.00%	0	0.00%	0	0.00%	1	20.00%
UTDBDS16.232	5	0	0.00%	0	0.00%	0	0.00%	1	20.00%
UTDBDS16.234	8	1	12.50%	0	0.00%	1	12.50%	2	25.00%
UTDBDS16.235	21	1	4.76%	0	0.00%	0	0.00%	2	9.52%
UTDBDS16.262	4	0	0.00%	0	0.00%	0	0.00%	1	25.00%
UTDBDS16.271	7	0	0.00%	0	0.00%	0	0.00%	1	14.29%
UTDBDS16.280	10	0	0.00%	0	0.00%	0	0.00%	4	40.00%
UTDBDS16.286	36	1	2.78%	3	8.33%	0	0.00%	8	22.22%
UTDBDS16.294	11	2	18.18%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.353	29	0	0.00%	0	0.00%	0	0.00%	5	17.24%
UTDBDS16.390	28	0	0.00%	0	0.00%	0	0.00%	21	75.00%
UTDBDS16.412	10	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.484	11	0	0.00%	0	0.00%	0	0.00%	1	9.09%
UTDBDS16.499	15	1	6.67%	0	0.00%	0	0.00%	2	13.33%
UTDBDS16.558	16	0	0.00%	0	0.00%	0	0.00%	2	12.50%
UTDBDS16.600	21	0	0.00%	0	0.00%	0	0.00%	16	76.19%
Total	857	16		9		5		168	

Table 6 – Continued

UT Smal pattern	Pattern Amounts	Wasatch Patterns	Wasatch % of Patterns	Washington Patterns	Washington % of Patterns	Wayne Patterns	Wayne % of Patterns	Weber Patterns	Weber % of Patterns
UTDBDS16.009	88	0	0.00%	7	7.95%	0	0.00%	6	6.82%
UTDBDS16.011	60	1	1.67%	4	6.67%	0	0.00%	2	3.33%
UTDBDS16.065	6	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.067	12	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.070	8	0	0.00%	0	0.00%	0	0.00%	1	12.50%
UTDBDS16.074	64	0	0.00%	0	0.00%	0	0.00%	4	6.25%
UTDBDS16.080	7	0	0.00%	0	0.00%	0	0.00%	1	14.29%
UTDBDS16.095	10	0	0.00%	1	10.00%	0	0.00%	0	0.00%
UTDBDS16.098	19	0	0.00%	1	5.26%	0	0.00%	3	15.79%
UTDBDS16.103	59	0	0.00%	1	1.69%	1	1.69%	5	8.47%
UTDBDS16.109	5	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.110	4	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.130	89	1	1.12%	11	12.36%	1	1.12%	6	6.74%
UTDBDS16.134	21	1	4.76%	0	0.00%	0	0.00%	1	4.76%
UTDBDS16.143	28	2	7.14%	1	3.57%	0	0.00%	3	10.71%
UTDBDS16.155	13	0	0.00%	1	7.69%	0	0.00%	1	7.69%
UTDBDS16.159	17	1	5.88%	1	5.88%	0	0.00%	2	11.76%
UTDBDS16.163	5	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.164	7	0	0.00%	0	0.00%	0	0.00%	3	42.86%
UTDBDS16.167	8	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.183	18	2	11.11%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.199	22	1	4.55%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.200	11	0	0.00%	0	0.00%	0	0.00%	1	9.09%
UTDBDS16.205	9	0	0.00%	0	0.00%	0	0.00%	1	11.11%
UTDBDS16.214	7	0	0.00%	0	0.00%	0	0.00%	1	14.29%
UTDBDS16.222	23	0	0.00%	3	13.04%	0	0.00%	2	8.70%
UTDBDS16.225	5	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.232	5	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.234	8	0	0.00%	1	12.50%	0	0.00%	0	0.00%
UTDBDS16.235	21	0	0.00%	0	0.00%	0	0.00%	1	4.76%
UTDBDS16.262	4	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.271	7	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.280	10	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.286	36	2	5.56%	2	5.56%	0	0.00%	0	0.00%
UTDBDS16.294	11	0	0.00%	1	9.09%	0	0.00%	2	18.18%
UTDBDS16.353	29	0	0.00%	0	0.00%	0	0.00%	4	13.79%
UTDBDS16.390	28	0	0.00%	0	0.00%	0	0.00%	0	0.00%
UTDBDS16.412	10	1	10.00%	1	10.00%	1	10.00%	0	0.00%
UTDBDS16.484	11	0	0.00%	0	0.00%	1	9.09%	2	18.18%
UTDBDS16.499	15	0	0.00%	0	0.00%	0	0.00%	1	6.67%
UTDBDS16.558	16	0	0.00%	2	12.50%	0	0.00%	0	0.00%
UTDBDS16.600	21	0	0.00%	2	9.52%	0	0.00%	0	0.00%
Total	857	12		40		4		53	
								Total	857

Table 7.

Reported Animal Exposure by County (Cases and Percent of Total Animal Group)

County	Birds	% of Total Birds	Canine	% of Total Canine	Cattle	% of Total Cattle	Cold-blooded Vertebrates	% of Total Cold-Blooded Animals	Feline	% of Total Feline	Poultry	% of Total Poultry	Random Animals	% of Total Random Animals	Ruminants (Noncattle)	% of Total Ruminants (Noncattle)	Simple Stomach	% of Total Simple Stomach	Vermin	% of Total Vermin
Beaver	1	3.13%	1	0.19%	0	0.00%	0	0.00%	1	0.37%	1	1.47%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
Box Elder	0	0.00%	11	2.12%	4	6.67%	0	0.00%	10	3.72%	2	2.94%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
Cache	2	6.25%	52	10.04%	15	25.00%	0	0.00%	38	14.13%	3	4.41%	0	0.00%	4	14.29%	7	12.73%	4	12.12%
Carbon	0	0.00%	0	0.00%	0	0.00%	0	0.00%	1	0.37%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
Davis	1	3.13%	40	7.72%	3	5.00%	1	6.25%	12	4.46%	3	4.41%	0	0.00%	1	3.57%	4	7.27%	2	6.06%
Duchesne	0	0.00%	0	0.00%	0	0.00%	0	0.00%	1	0.37%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
Garfield	0	0.00%	3	0.58%	0	0.00%	0	0.00%	1	0.37%	1	1.47%	0	0.00%	1	3.57%	0	0.00%	0	0.00%
Grand	0	0.00%	1	0.19%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
Iron	0	0.00%	12	2.32%	0	0.00%	1	6.25%	11	4.09%	3	4.41%	0	0.00%	1	3.57%	1	1.82%	2	6.06%
Juab	1	3.13%	5	0.97%	2	3.33%	0	0.00%	5	1.86%	1	1.47%	0	0.00%	0	0.00%	1	1.82%	0	0.00%
Kane	0	0.00%	2	0.39%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
Millard	1	3.13%	10	1.93%	1	1.67%	1	6.25%	7	2.60%	1	1.47%	0	0.00%	2	7.14%	1	1.82%	2	6.06%
Morgan	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	1	3.03%
Piute	0	0.00%	2	0.39%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%

Table 7 – Continued

County	Birds	% of Total Birds	Canine	% of Total Canine	Cattle	% of Total Cattle	Cold-blooded Vertebrates	% of Total Cold-Blooded Animals	Feline	% of Total Feline	Poultry	% of Total Poultry	Random Animals	% of Total Random Animals	Ruminants (Noncattle)	% of Total Ruminants (Noncattle)	Simple Stomach	% of Total Simple Stomach	Vermin	% of Total Vermin
Rich	0	0.00%	2	0.39%	1	1.67%	0	0.00%	1	0.37%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
Salt Lake	11	34.38%	186	35.91%	13	21.67%	3	18.75%	88	32.71%	22	32.35%	2	66.67%	6	21.43%	17	30.91%	5	15.15%
San Juan	0	0.00%	1	0.19%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
Sanpete	0	0.00%	11	2.12%	0	0.00%	1	6.25%	5	1.86%	3	4.41%	0	0.00%	0	0.00%	3	5.45%	0	0.00%
Sevier	1	3.13%	13	2.51%	3	5.00%	0	0.00%	12	4.46%	4	5.88%	0	0.00%	1	3.57%	0	0.00%	3	9.09%
Summit	0	0.00%	10	1.93%	1	1.67%	0	0.00%	2	0.74%	2	2.94%	0	0.00%	1	3.57%	0	0.00%	1	3.03%
Tooele	0	0.00%	6	1.16%	0	0.00%	0	0.00%	4	1.49%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	1	3.03%
Uintah	0	0.00%	2	0.39%	0	0.00%	0	0.00%	2	0.74%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
Utah	8	25.00%	77	14.86%	8	13.33%	7	43.75%	38	14.13%	16	23.53%	0	0.00%	5	17.86%	10	18.18%	8	24.24%
Wasatch	0	0.00%	12	2.32%	3	5.00%	1	6.25%	5	1.86%	0	0.00%	0	0.00%	2	7.14%	3	5.45%	1	3.03%
Washington	4	12.50%	21	4.05%	2	3.33%	0	0.00%	8	2.97%	5	7.35%	0	0.00%	1	3.57%	3	5.45%	0	0.00%
Wayne	0	0.00%	0	0.00%	0	0.00%	0	0.00%	1	0.37%	0	0.00%	0	0.00%	1	3.57%	0	0.00%	0	0.00%
Weber	2	6.25%	38	7.34%	4	6.67%	1	6.25%	16	5.95%	1	1.47%	1	33.33%	2	7.14%	5	9.09%	3	9.09%
Total	32	100%	518	100%	60	100%	16	100%	269	100%	68	100%	3	100%	28	100%	55	100%	33	100%

Table 8.

Animal Results

Animal Group	n =	Obtained Value
Bird	25	$r_{(41)} = 0.521, p < .01$
Canine	331	$r_{(41)} = 0.915, p < .01$
Cattle	48	$r_{(41)} = 0.481, p < .01$
Cold-blooded Vertebrates	12	$r_{(41)} = 0.534, p < .01$
Feline	174	$r_{(41)} = 0.906, p < .01$
Poultry	45	$r_{(41)} = 0.516, p < .01$
Random Animals	1	$r_{(41)} = 0.279, p > .01$
Ruminants	21	$r_{(41)} = 0.644, p < .01$
Simple Stomach	37	$r_{(41)} = 0.669, p < .01$
Vermin	22	$r_{(41)} = 0.756, p < .01$

Table 9.

County Results

Utah Counties	n =	Obtained Value
Beaver	7	$r_{(40)} = 0.279, p > .01$
Box Elder	19	$r_{(40)} = 0.808, p < .01$
Cache	131	$r_{(40)} = 0.826, p < .01$
Carbon	2	$r_{(40)} = -0.020, p > .01$
Davis	33	$r_{(40)} = 0.733, p < .01$
Duchesne	2	$r_{(40)} = 0.364, p > .01$
Garfield	4	$r_{(40)} = 0.454, p < .01$
Grand	1	$r_{(40)} = 0.019, p > .01$
Iron	15	$r_{(40)} = 0.649, p < .01$
Juab	10	$r_{(40)} = 0.779, p < .01$
Kane	2	$r_{(40)} = 0.511, p < .01$
Millard	19	$r_{(40)} = 0.671, p < .01$
Morgan	0	NA
Piute	1	$r_{(40)} = 0.325, p > .01$
Rich	2	$r_{(40)} = 0.566, p < .01$
Salt Lake	268	$r_{(40)} = 0.877, p < .01$
San Juan	0	NA
Sanpete	19	$r_{(40)} = 0.657, p < .01$
Sevier	15	$r_{(40)} = 0.715, p < .01$
Summit	16	$r_{(40)} = 0.391, p > .01$
Tooele	9	$r_{(40)} = 0.480, p < .01$
Uintah	5	$r_{(40)} = 0.217, p > .01$
Utah	168	$r_{(40)} = 0.682, p < .01$
Wasatch	12	$r_{(40)} = 0.266, p > .01$
Washington	40	$r_{(40)} = 0.777, p < .01$
Wayne	4	$r_{(40)} = 0.338, p > .01$
Weber	53	$r_{(40)} = 0.789, p < .01$

CHAPTER IV

DISCUSSION

The aim of this study was to describe the surveillance of *Campylobacter* infection in Utah from 2002 through 2010. *Campylobacter* has emerged since the 1980s to become the largest cause of gastric enteritis worldwide, and yet the source of most cases is unknown (1, 23, 34, 38, 45). Without the complete knowledge of *Campylobacter* sources and modes of transmission, it is unlikely that infection rates will stop increasing (17). A new perspective may be useful in uncovering a previously hidden but important source of *Campylobacter* infection (8). This study attempted to gather a large amount of laboratory and epidemiologic data and combine the two, so future information can be added and hopefully lead to better *Campylobacter* source detection in the future.

Interpretation

This study focused on collecting epidemiological information, specifically reported animal exposures and patient county of residence, and combined this with PFGE results for *Campylobacter* infections from 2002-2010. As expected, based on population density, the majority of the data collected involved patients

in Salt Lake County. Cache County's *Campylobacter* incidence is surprising when comparing the percent of total *Campylobacter* cases to the percent of total Utah population. Cases in Cache County are over three times higher than the expected percentage based on its population (Table 1). With other counties not exhibiting such an extreme difference, Cache County stands out and creates speculation into the cause. One cause may be frequent human exposure to several different animal groups as a consequence of everyday life in Cache County. Cattle are common in Cache County and are the fourth most common *Campylobacter* exposure in the state (Table 7). As population size was not figured into Table 7, the percentage of animal group per county would most likely be even larger for smaller counties such as Cache. One of the leading agriculture counties in Utah, Cache County contains a more diverse animal population than that of more urban settings. Animals known to carry and shed *Campylobacter*, such as cattle and sheep, have more interaction with people in Cache County than in Salt Lake County. All of these conditions may help uncover the spike in *Campylobacter* cases in Cache County compared to other less agricultural counties.

Davis County's results are also surprising. Unlike Cache County, Davis County case numbers are less than half of what is expected based on the population (Table 1). State and local epidemiologists have noticed this lack of *Campylobacter* infections in Davis County and have tried to understand the difference. Davis County residents may be protected to some degree because no raw milk is sold in their county. It is also interesting to note that although Davis is

the third largest county in Utah, the reported animal exposure data lacks any strong support connecting any animal group to Davis County (Table 7). The greatest percentage Davis County held in all 10 animal groups was with the Canine group representing 7.72% of the total reported canine exposure in Utah. This percentage was not outstanding as Cache County, a smaller county, reported a higher percentage along with Utah and Salt Lake Counties. The data gathered in Table 7 show a relatively low reported animal exposure percentage in all animal groups. This may suggest a possible reason for the lower than expected incidence of *Campylobacter* in Davis County.

The data gathered in this study also showed the power of larger sample sizes. Of the 29 total Utah counties, only Cache, Salt Lake, and Utah Counties showed a pattern variability of less than 50%. Salt Lake and Utah Counties represent the two highest populated counties in Utah with Cache County the sixth. These results may indicate that more dense populations will acquire *Campylobacter* infections more commonly from the same source. Alternately, it may indicate that smaller, more rural populations have many different infection opportunities because of their proximity to many different animal groups and possibly less human interactions that could potentially spread disease.

Lower pattern variability was also shown involving the two most common animal groups, canines and felines. Overall, dogs and cats make up over 70% of all total reported animal exposures. Again the top two animal groups exhibit the only pattern variability under 50%. This raises the same questions posed by the larger counties with lower variability. Overall, it seems that among the animal

groups, those represented by canines and felines typically are considered to be more than pets but an extension of family for some. Although some birds (e.g. cockatiel, parakeets and parrots) and cold-blooded animals (e.g. fish, frogs, lizards, and snakes) are considered house pets, handling and interactions that may result in *Campylobacter* infection seem less common. Many cats and dogs are let free to roam inside homes and are often found in kitchens while food preparations are occurring. A study from the United Kingdom found that the majority of PFGE patterns found in dogs were also isolated from humans (33). Lifestyles that integrate pets in all aspects of living may enable *Campylobacter* more opportunities for infection.

The obtained values as seen in Table 8 show that the common PFGE patterns and all animal groups, except Random Animals, are related. As the obtained values for all animal groups except Random Animals exceeds that of the critical value, the null hypothesis can be rejected. The animals included in groups Birds, Canine, Cattle, Cold-Blooded Vertebrates, Feline, Poultry, Ruminants, Simple Stomach, and Vermin exhibit a significant relationship by having obtained values of greater than the critical value of 0.3932. This association does not necessarily indicate causation. The Canine and Feline animal groups show very strong relationship with each having over 80% of their variance in common patterns accounted. Cattle showed the lowest relationship of those that exceeded the critical value with an obtained value of 0.481. Here, Cattle exhibit a moderate relationship where 23% of the variance is accounted for

leaving 77% of variance unaccounted. Although the statistics are significant at the 0.01 level, they are less meaningful the lower the obtained value falls.

As for the counties obtained value, only Cache, Box Elder and Salt Lake show a very strong relationship. Cache County shows the strongest relationship with 77% of the PFGE pattern variance accounted for but still leaving 23% of the patterns unexplainable. Sixteen out of the 29 counties have a significant relationship at the 0.01 level between common PFGE patterns and the county of residence. As was seen with the Animal Groups correlation, the closer the obtained value is to the critical value in the County Results Table, the less meaningful the association. Overall, more than half of the counties in Utah have correlation coefficients that support the research hypothesis.

Limitations

Several obstacles prevent better understanding of the epidemiology of *Campylobacter.* Since *Campylobacter* has emerged, researchers have marveled at the genetic heterogeneity that is so unusual among enteric pathogens (7, 16, 27, 29, 39, 46). This genetic instability strengthens the belief that one PFGE pattern results from a common source (7). Many outbreaks involving *Campylobacter* have included more than one PFGE pattern although the epidemiology has confirmed their single source of contamination (7, 29, 40). This instability has caused some scientists to be wary of implementing PFGE as an epidemiologic tool because of the amount of investigation that is required to pinpoint a single source contaminate when multiple patterns are seen (49).

Much of what is involved in performing PFGE poses potential difficulties for many situations. To begin with, PFGE is not a rapid test. Typically PFGE can be broken down into three different days of laboratory work required for results (17). Each of the three days involves multiple, precise steps to finish with an analyzable pattern at the end of day three. If the protocol is not followed exactly or the sample is not healthy, the three days will have been spent in vain. *Campylobacter* is much more fastidious than other enteric bacteria and requires more diligence and attention. This large time investment for potentially no results is somewhat off-putting for many researchers (40).

Even before PFGE can be performed, the organism must be isolated and contain enough growth to create a heavy cell suspension, sometimes requiring up to 48 hours (30). Adding this long incubation time to an already lengthy laboratory method means investigators may ask patients to recall possible exposure information sometimes weeks following infection. This time delay, combined with insufficient resources for investigation, leaves little chance at finding common exposures and therefore remediation (21). The lack of a single, widely used typing scheme has also hindered the advancement in understanding *Campylobacter's* natural reservoirs and preventing further transmission (21, 30).

The cost involved with PFGE creates another limitation. PFGE requires very specialized equipment (18). The laboratories involved in PulseNet all possess a CHEF Mapper system which is made up of three main parts: a power module, cooling module, and electrophoresis chamber. The amount of CHEF Mapper systems a laboratory might have, can directly affect the length of time

involved in producing *Campylobacter* PFGE results, delaying source identification even more. The USLPH has three CHEF Mapper systems but runs PFGE according to a disease hierarchy where *Campylobacter* is considered the least critical, which may also delay results. A molecular imaging system is also required along with software capable of analyzing PFGE patterns and their relatedness. All of this specialized equipment is expensive and space consuming. Other laboratory equipment, including water baths, turbidity meters, plug molds, gel molds, gel levelers, and computers, are also necessary. Including only supplies and reagents, such as several different types of disposable tubes, gel agarose, buffers, and very expensive restriction enzymes, bring a *Campylobacter* gel cost to over $100 each. This does not include the cost for the equipment and maintenance. USLPH has not received funding for PFGE for several years and so only runs full *Campylobacter* gels that may require personnel to wait for enough samples to be received, delaying results significantly depending on the season. These cost-related delays in PFGE results may have significantly inhibited patient's animal exposure recall during their case investigation affecting accurate data collection. The US annual estimated cost of *Campylobacter* in the 1990s was approximately $4.3 billion (23). Considering how health care costs have risen over the years, one can only imagine how this number may have increased.

Culturing techniques may limit the amount and type of *Campylobacter* cases. Many *Campylobacter* samples are thought to be excluded because of differing growth requirements. Some *Campylobacter* species only grow at 37ºC,

but research shows that *Campylobacter's* transition to the nonculturable state is heightened at this temperature potentially leading to false negatives (38). When a *Campylobacter* is isolated, it can be difficult to determine if the patient is co-infected. Most laboratory isolation methods look for only one positive colony. Certain source outbreaks contain more than one strain of *Campylobacter* and can co-infect a single host (37).

PFGE also has some intrinsic limitations specific to its methodology. *Campylobacter's* unusual genetic instability has been known to confuse many outbreak investigations. Mutations during outbreaks that change the PFGE pattern can lead to epidemiological misinterpretation of the data and therefore misinterpretation of the cause (40, 46). *Campylobacter* PFGE patterns typically contain less than ten bands, significantly less than other enteric bacteria patterns. Some feel that this lack of bands is thought to limit the discriminatory power of *Campylobacter* PFGE (40). The restriction enzymes used can also pose some constraints to the ability of PFGE. The first enzyme *SmaI*, used in PulseNet, is thought to be more of a grouping enzyme while the second enzyme *KpnI*, was found to be more discriminatory (16, 40). Funding and personnel restraints, however, limit the use of two enzymes in testing *Campylobacter* so that very little testing with *KpnI* is performed.

With the previous limits considered, this study acknowledges additional limitations. The epidemiologic information that was collected and added to the PFGE database involved many investigators across the state, many different epidemiologists, many different versions of the investigation case report forms,

and several public health databases spanning nine years. These systematic differences may have affected the data in unknown ways. An interviewer's diligence in collecting reported animal exposure data may differ from person to person. Some may just ask for any known animal exposures while others may have asked about each individual animal. There is also the threat of selection bias. This study relied completely upon infected people visiting their doctor, their doctor asking for a stool sample, and ultimately, the patient submitting that stool sample (49).

Among those samples that are submitted to the USLPH, certain considerations must be kept in mind. Some patients visit doctors multiple times during their *Campylobacter* infection and some submit multiple stool samples. As all *Campylobacter* samples are required to be submitted to the public health laboratory, the potential to receive multiple samples per single infection is very real. This, along with single source outbreaks resulting in several indistinguishable PFGE patterns, can skew the data collected. It is also important to keep in mind that this study included epidemiological data recorded as reported animal exposure. This does not necessarily mean that the *Campylobacter* infection was caused by said animal, only that the patient remembered and reported an animal encounter. Also, this study recognizes the importance sample size has on data results (35). The low incidence of *Campylobacter* among the smaller population counties and animal groups with few representative cases leaves questions unanswered that may have been explained by a larger sample size.

Conclusions

This study was able to combine data involving the epidemiological and laboratory side of *Campylobacter* infections that has previously been kept separate. Combining the two aspects involved in *Campylobacter* infections allows for a broader spectrum to be understood and more pieces of the puzzle to be visualized. In order for this study to help make progress in identifying more sources of *Campylobacter* infection, more stool samples need to be submitted and better data collected. A larger sample population can only increase our knowledge of how *Campylobacteriosis* occurs in Utah. With more information, it is possible to better understand *Campylobacter* and slow its emergence. Prevention strategies have been successful in the past. Data produced by PulseNet has helped decrease the number of enteric outbreaks nationally since its development. If all states mandated *Campylobacter* submission and routinely performed PFGE, much more about *Campylobacter's* source would be known. History has shown it to be reasonable to expect cases to drop if *Campylobacter* was routinely investigated. Funding needs to be provided for public health prevention. Without it, *Campylobacter* appears to be unstoppable.

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